and *zic* families are among the earliest markers of the presumptive neural crest and their expression is first seen during gastrulation at the border of the neural plate. The *pax3/7* and *zic* genes have undergone multiple duplications in the evolution of teleost fish. Some of these duplications occurred soon after the origin of vertebrates, while others are teleost-specific. While many *pax3/7* and *zic* paralogs are expressed during neural crest development, their expression is initiated at different times and is seen in different areas along the anterior–posterior axis. These expression differences could result from the gain or loss of regulatory elements in particular *pax3/7* and *zic* genes or from modification of existing elements. To distinguish between these possibilities, we identified multiple paralogous *pax3/7* and *zic* neural plate border enhancers from the *Fugu* genome and characterized them in transgenic zebrafish. We found that the paralogous enhancers share sequence similarity, are similarly positioned relative to the coding sequence and we provide evidence that many are directly regulated by Wnt signaling. Despite these similarities, the enhancers have undergone spatial and temporal sub-functionalization, with some paralogs only driving gene expression later in neural crest development or only in sub regions of the neural plate border. This indicates that differences in expression among neural plate border genes are the result of modifications to existing enhancers.

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Program/Abstract # 414

Developmental switch of primary sensory system from Rohon–Beard cells to Dorsal root ganglia

Kiyoshi Kawakami[a], Hiroshi Yajima[a], Makoto Suzuki[b], Haruki Ochi[c], Keiko Ikeda[a], Shigeru Sato[a], Hajime Ogino[c], Naoto Ueno[b]
[a]*Div. of Biol. Centr. Mol. Med. JMU, Tochigi, Japan*
[b]*Div. of Morpho. NIBB. Aichi, Japan*
[c]*Grad. Sch. Biol. Sci. NAIST, Nara, Japan*

Primary sensory system is composed of two groups of cells that reside intra- and extra-spinal cord in vertebrates. The former is Rohon–Beard (RB) cells, which are observed in fish embryo and amphibian larvae but not in birds and mammals. The latter represents dorsal root ganglia (DRG), which mediate a variety of sensations in the body. In fish and amphibia, RB cells undergo cell death by apoptosis followed by the formation of DRG at later stages. *Six1* is not expressed in RB cells until the onset of their apoptosis and expressed in neurons of DRG. In *Six1/Six4* double knockout mice (dKO), ectopic sensory neuron-like cells positive for Islet1/2 in conjunction with Tlx3 and Kv1.1, specific markers for RB cells, were observed in the spinal cord. Axons extended to outside of the spinal cord from the ectopic cells. These features suggest that the ectopic cells appeared in the spinal cord of dKO correspond to RB cells, which are never observed in the wild type. These results allow us to hypothesize that acquisition of the expression of *Six1* in early developmental stage of amniotes causes loss of RB cells and promotes formation of DRG. To address this possibility, we overproduced *Six1* protein in *Xenopus* embryos. The number of RB cells was decreased and extra-spinal ectopic sensory neurons appeared. We also found that a mouse *Six1* enhancer specific for DRG showed activity in dorsal neural tube in *Xenopus* larvae, while corresponding *Xenopus* enhancer has no such activity.

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Program/Abstract # 415

Characterization of late emerging trunk neural crest cells in the turtle *Trachemys scripta*

Judith A. Cebra-Thomas[a], James Robinson[b], Melinda Yin[b], James McCarthy[a], Sonal Shah[a], Scott F. Gilbert[b]
[a]*Department of Biology, Millersville University, Millersville, PA, USA*
[b]*Department of Biology, Swarthmore College, Swarthmore, PA, USA*

Turtle plastron bones develop by intramembranous ossification from the condensation of cells that stain positively for HNK1, PDGFRα and p75, indicating that these bones are derived, like the facial bones, from neural crest cells. At Greenberg stage 17, comparable to H&H Stage 28 chick embryos and well after the initial wave of neural crest migration, cells that are positive for HNK1 and the early neural crest marker, FoxD3 begin accumulating in the thickened dermis of the carapace and migrating to the developing plastron. These cells possess the defining attribute of neural crest cells, that of emerging from the neural tube. However they emerge from the neural tube in a second, later wave. HNK1+ cells were observed migrating away from cultured neural tubes from St.17 embryos. When the lipophilic dye DiI was injected into the lumen of the neural tube of St.17 turtle embryos, DiI-positive cells were observed in the carapacial ridge neural crest "staging area" within a day. These data support our hypothesis that the plastron bones of the turtle are formed by a late emerging population of neural crest cells that collect dorsally in the carapacial dermis and then migrate ventrally. Currently, we are in the process of comparing the molecular and functional properties of these late trunk neural crest cells with those of early trunk and cranial neural crest cells.

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Program/Abstract # 416

Brd2 knock-down in zebrafish results in morphological defects, abnormal patterns of mitosis and apoptosis, and misregulation of midbrain/hindbrain gene markers

Giana J. Bistany, Eliza Fradkin, Heather Melville, Catharine Comstock, Angela J. DiBenedetto
Department of Biol., Villanova Univ., Villanova, PA, USA

Brd2 is an epigenetic transcriptional co-regulator involved in control of mitosis and apoptosis in mammalian cells, and homeotic gene expression in *Drosophila*. Zebrafish Brd2 RNA is restricted in somitogenesis to central nervous system and gut, where homeotic genes control segmentation. Morpholino knock-down of Brd2 results in morphological defects, including midbrain/hindbrain boundary (MHB) blurring, reduced hindbrain volume, and disorganized notochord and somites. These defects are likely due to misregulation of processes known to be under Brd2 control. Consequently, Brd2 morphants were analyzed for levels and patterns of apoptosis, cell proliferation, and homeotic gene expression using TUNEL assay and BrdU incorporation followed by fluorescent confocal microscopy, and *in situ* hybridization, respectively. Brd2 morphants exhibit a severe reduction in apoptosis in early somitogeneis, and an opposite dramatic increase in apoptosis at later stages, implying bimodal regulation of cell death by Brd2. Although levels of proliferation appear similar in knock-down and control embryos, patterns of proliferating cells in morphants are disorganized, especially in developing somites, consistent with gross morphological defects. Lastly, *engrailed2a*, a homeotic MHB marker, is absent or severely reduced in its caudal domain, suggesting defective hindbrain patterning of morphants. Thus, Brd2 is critical for the proper balance of cell death and proliferation, and likely

plays a conserved role as a homeotic regulator, during vertebrate development.

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Program/Abstract # 417
Integrative imaging of the developing opossum cochlea
Lisa Noelle Cooper, Karen E. Sears
Department of Animal Bio., Univ. of Illinois, Urbana, IL 61801, USA

The short-tailed opossum (*Monodelphis domestica*) is a marsupial mammal that gives birth to highly underdeveloped young that complete much of their sensory development outside the womb while fused to the mother's teat. While suckling, the primary organ of hearing, the cochlea, undergoes an extraordinary morphological transition from a cylinder to a coiled cochlea with 1.9 turns. Because this transition occurs *ex utero*, opossum cochlear development can be experimentally manipulated *in vivo*, making the opossum an ideal model for inner ear development. This is important, as the genetic underpinnings of cochlear morphogenesis are largely unknown. This study utilizes the opossum as a novel mammalian model for cochlear development, with the aim of synthesizing developmental morphogenetic and molecular signaling data to pinpoint mechanisms shaping mammalian cochlear development. High resolution computed tomography (CT) and magnetic resonance imaging (MRI) technologies allowed visualization of cochlear outgrowth and coiling. Comparisons with histological sections and cleared and stained pups indicated that MRI scans more accurately differentiated soft tissue boundaries, and these data were used to reconstruct a 3D model of opossum cochlear development. Central toward understanding cochlear outgrowth is pinpointing regions of cell proliferation and apoptosis. Apoptosis assays indicated that cell death occurred along the base of the developing cochlear coils, while proliferation (phosphohistone-H3) preferentially occurred along their lateral margins. Taken together, these results lay the foundation for future utilization of the opossum as a novel model for mammalian inner ear morphogenesis.

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Program/Abstract # 418
A new model for the evolution of the vertebrate jaw
Daniel M. Medeiros[a], Jacob Doherty[a], Maria V. Cattell[a], Tatjana Sauka-Spengler[b], Marianne Bronner-Fraser[b], Feiqiao Yu[b], Robert Cerny[c]
[a]*EBIO Department, Univ. of Colorado, Boulder, CO, USA*
[b]*Div. of Biol., CALTECH, Pasadena, CA, USA*
[c]*Department of Zool., Charles University in Prague, Czech Republic*

The appearance of jaws was a turning point in vertebrate evolution because it allowed primitive vertebrates to capture and process large, motile prey. The vertebrate jaw consists of separate dorsal and ventral skeletal elements connected by a joint. How this structure evolved from the unjointed gill bars of a jawless ancestor is an unresolved question in vertebrate evolution. To understand the developmental bases of this evolutionary transition, we examined the expression of 12 genes involved in vertebrate pharyngeal patterning in the jawless fish, lamprey. Contrary to previous reports, we find nested expression of *Dlx* genes, and combinatorial expression of *Msx*, *Hand* and *Gsc* genes along the dorso-ventral (DV) axis of the lamprey pharynx, indicating gnathostome-type pharyngeal patterning evolved before the appearance of the jaw. In addition, we find that Bapx and Gdf5, key regulators of joint formation in gnathostomes, are not expressed in the lamprey first arch, while Barx, which is absent from the intermediate first arch in gnathostomes, marks this domain in lamprey. Taken together, these data support a new scenario for jaw evolution in which recruitment of Bapx and Gdf5 into a pre-existing DV patterning program drove the evolution of the jaw by altering the identity of intermediate first arch chondrocytes. We present this "Pre-pattern/Cooption" model as an alternative to current models linking the evolution of the jaw to the evolution of novel pharyngeal DV pattern.

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Program/Abstract # 419
The role of neural crest progenitor population specification and proliferation dynamics in establishing species-specific differences in jaw size
Jennifer L. Fish, Rich A. Schneider
Department of Orthopaedic Surgery, University of California at San Francisco, San Francisco, CA, USA

The diversification and adaptive success of vertebrates owes a great deal to their specialized feeding apparatuses. The jaw skeleton derives from the cranial neural crest (CNC), a population of cells unique to vertebrates. Despite its basic developmental conservation, the adult jaw varies tremendously in both size and shape. Recently, the orchestration of developmental programs regulating jaw size and shape has been shown to be under the control of CNC cells. Yet, underlying molecular and cellular mechanisms driving species-specific changes in jaw size remain unknown. To test the hypothesis that CNC progenitor population number and proliferation rates contribute to species-specific differences in jaw size, we compare CNC development in two morphologically distinct birds, duck and quail. We analyze expression of genes involved in neural tube regionalization including *Otx2*, *Foxg1*, *Fgf8*, and *Krox20*, and genes involved in the induction and maintenance of CNC such as *Pax7*, *FoxD3*, and *Sox10*, in duck and quail embryos at Hamburger and Hamilton (HH) stages 4–12. These stages span the period of time when CNC become specified and emigrate from the rostral neural tube. We also compare proliferation rates in duck and quail premigratory CNC and postmigratory mandibular mesenchyme, which show that duck CNC proliferate more slowly than those of quail. Our results indicate that molecular and cellular differences emerge early on during duck and quail development, which likely contribute to species-specific variation in jaw size.

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Program/Abstract # 420
Evolution of vertebrate skeletal myogenesis: Insights from the cyclostome lamprey
Rie Kusakabe[a,b], Shigehiro Kuraku[c], Shigeru Kuratani[b]
[a]*Department of Biol., Grad. Sch. Sci., Kobe Univ., Japan*
[b]*Lab. for Evol Morph., CDB, RIKEN, Japan*
[c]*Dept. Biol., Univ. of Konstanz, Germany*

Skeletal muscles of gnathostomes (jawed vertebrates) are categorized into epaxial and hypaxial groups morphologically separated at the level of the notochord. During development, portions of the hypaxial dermomyotome undergo delamination to provide migratory myoblasts that give rise to the tongue muscles, the tranpezius (cucullaris) muscles, and the limb muscles. These muscles require activation of specific developmental genes, such as MRFs, Pax3 and Lbx1, at the ventral (hypaxial) side of the dermomyotome. To gain